

Viscoelastic Properties of Kenaf Bast Fiber in Relation to Stem Age

Abstract Natural fibers traditionally used for cordage are proving valuable for advanced industrial applications due in part to beneficial physical and chemical properties, but also because they are a renewable and biodegradable resource. Kenaf (*Hibiscus cannabinus* L., Malvaceae) produces high yields of lignocellulosic bast fibers in the bark layer, and is a promising crop for supplying emerging fiber markets. Bast fibers are bundles of cells that undergo extensive cell-wall thickening during maturation. Bundle maturity is therefore an important determinant of the fibers' mechanical properties and ultimately contributes to their quality in specific applications. Fiber bundles in stem sections of progressive age were analyzed by epifluorescence microscopy, and viscoelastic properties determined by dynamic mechanical thermal analysis. Early-forming primary fibers were larger than later-forming secondary fibers, but cell-wall thickening contributed most to elastic and viscous response of the fiber.

Key words fabric formation, fiber, performance, processing, properties, strength, yarn

Kenaf (*Hibiscus cannabinus* L., Malvaceae) is a high-yielding tropical plant traditionally grown for the long, strong bast fibers that develop in the bark layer of the stem. Cultivation spread internationally in the early to mid-twentieth century, but interest waned, particularly in developed countries, as raw materials for cordage and related products shifted from biological to petrochemical sources [1]. Concerns over rising costs, unstable supply, and negative environmental impact of fossil fuels are promoting renewed interest in traditional fiber crops [2].

Kenaf is a multipurpose crop with various harvestable components: leaves and tender shoots are suitable for forage; the woody core has attributes for forest-product substitutes, absorbents, and structural materials; and seeds have an oil and protein composition similar to cotton seed [3]. The bast fibers, however, remain the primary economic

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incentive to grow kenaf. Beyond cordage, bast fibers are expanding into new markets of moldable, nonwoven fabrics, and reinforced composite materials in automotive, aerospace, packaging and other industrial applications [4, 5]. This trend is in part due to the fiber's physical properties of light weight, competitive tensile strength and stiffness, and vibration damping properties, and also due to the fiber being a renewable and biodegradable resource [2, 4]. Nonwoven materials made of kenaf or other natural fibers blended with polyester or polypropylene are efficient sound absorbers and can meet industry specifications of

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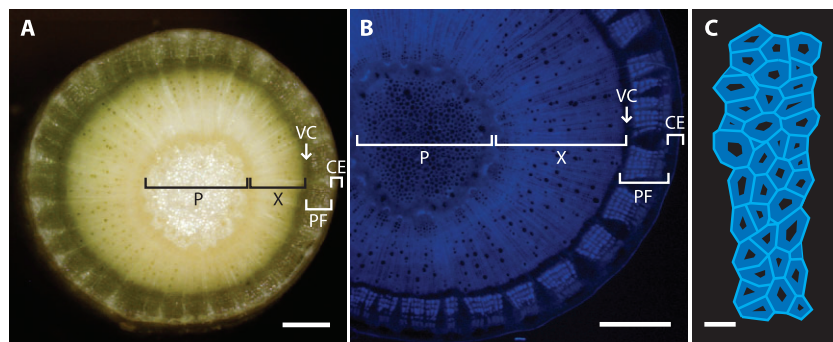


Figure 1 Kenaf stem cross-sections. (A) Mature kenaf stem photographed under white light with a dissecting microscope. (B) A section of the same stem photographed with UV epifluorescence to emphasize lignified cells. P, pith; X, xylem; VC and arrow, vascular cambium; PF, phloem fiber bundles; CE, cortex and epidermis. Scale bars in A and B, 1 mm. (C) Schematic diagram of a primary phloem fiber bundle. Light blue represents lignin in the primary cell walls and middle lamella, dark blue is lignin in the secondary walls, and black is the hollow lumen. Scale bar approximates 20 μ m.

flammability, and odor and mildew resistance [4, 5]. Mueller and Krobjilowski [6] studied the formation of composites of flax fibers and biodegradable, melt-blown polymeric materials, and observed that they have many properties comparable to polypropylene based composites.

Bast fibers are bundles of specialized cells called sclerenchyma fibers that have extensive secondary cell wall thickenings and are usually dead at maturity [7]. Sclerenchyma fibers can be classified by the tissues in which they are found; in kenaf the sclerenchyma fibers are part of the phloem tissue and may be referred to as phloem fibers (Figure 1). The main role of phloem fibers is to provide protection and mechanical support [8]. Fiber bundles may differentiate from cells derived from the procambium during primary growth (growth from apical meristems), or from cells of the vascular cambium during secondary growth (growth from lateral meristems that contribute to plant girth). Previous studies have termed them as primary and secondary fibers, respectively [9]. With continued secondary growth, derivatives of the vascular cambium alternate between differentiating into phloem fibers or phloem conducting cells and associated phloem parenchyma [7].

The cell walls of kenaf phloem fibers are composed of cellulose and noncellulosic substances such as hemicelluloses, pectins, and lignins [10]. Lignin is a complex aromatic polymer that forms strong hydrogen bonds with the carbohydrate components of cell walls, and along with pectins, cement the individual fiber cells together in the fiber bundle [11, 12]. Lignin in the fiber cells is readily detected with ultraviolet light since the aromatic ring fluoresces blue [13], and is predominantly found in secondary cell walls that begin to form after cell expansion has ceased. Secondary cell wall thickening continues as the bundle matures, occupying more and more of the space

occupied by the cell cytoplasm, until only a small central lumen remains [8]. Cotton fibers, in contrast, are the cell wall remnants of single cells that grow on the surface of the cotton seed, and consist of virtually pure cellulose [14]. Cotton fibers also have a central lumen, but the cell walls collapse in on it to produce flattened tubes, and in cross-section, cotton fibers have a kidney-bean shape [14]. Kenaf bast fibers, on the other hand, are composed of bundles of cells that retain their shape to create a honeycomb-like structure when viewed in cross-section (Figures 1, 2, and 3).

Retting is the separation of the fiber from other tissues, and is based on the differential degradation of fiber and non-fiber cells. The extensive cell wall thickening of fiber cells makes them exceptionally more stable. Retting is labor and resource intensive, and is a limiting factor in bast fiber production. Kenaf fiber is commonly retted chemically, enzymatically with partially purified enzyme preparations, or biologically with micro-organisms and water [15, 16]. Each process has advantages and disadvantages, and affects the composition of the fiber. Natural retting with micro-organisms can be time consuming and produce fiber of inconsistent quality, but is inexpensive and is by far the most widely used method worldwide. Because this method is based on the natural decomposition of the plant material, it is arguably the most environmentally benign, but is also water-use intensive and low oxygen levels in stagnant water can result in methane and other undesired gasses [17]. However, when properly managed, retting can be carried out in flooded rice fields, and benefit the crop by leaving behind nutrients, or can be combined with fish-farming operations [17]. The consistency of naturally retted fiber is sufficient for traditional uses, however, new application may require additional control over the process. Retting with enzyme preparations is faster and more consistent

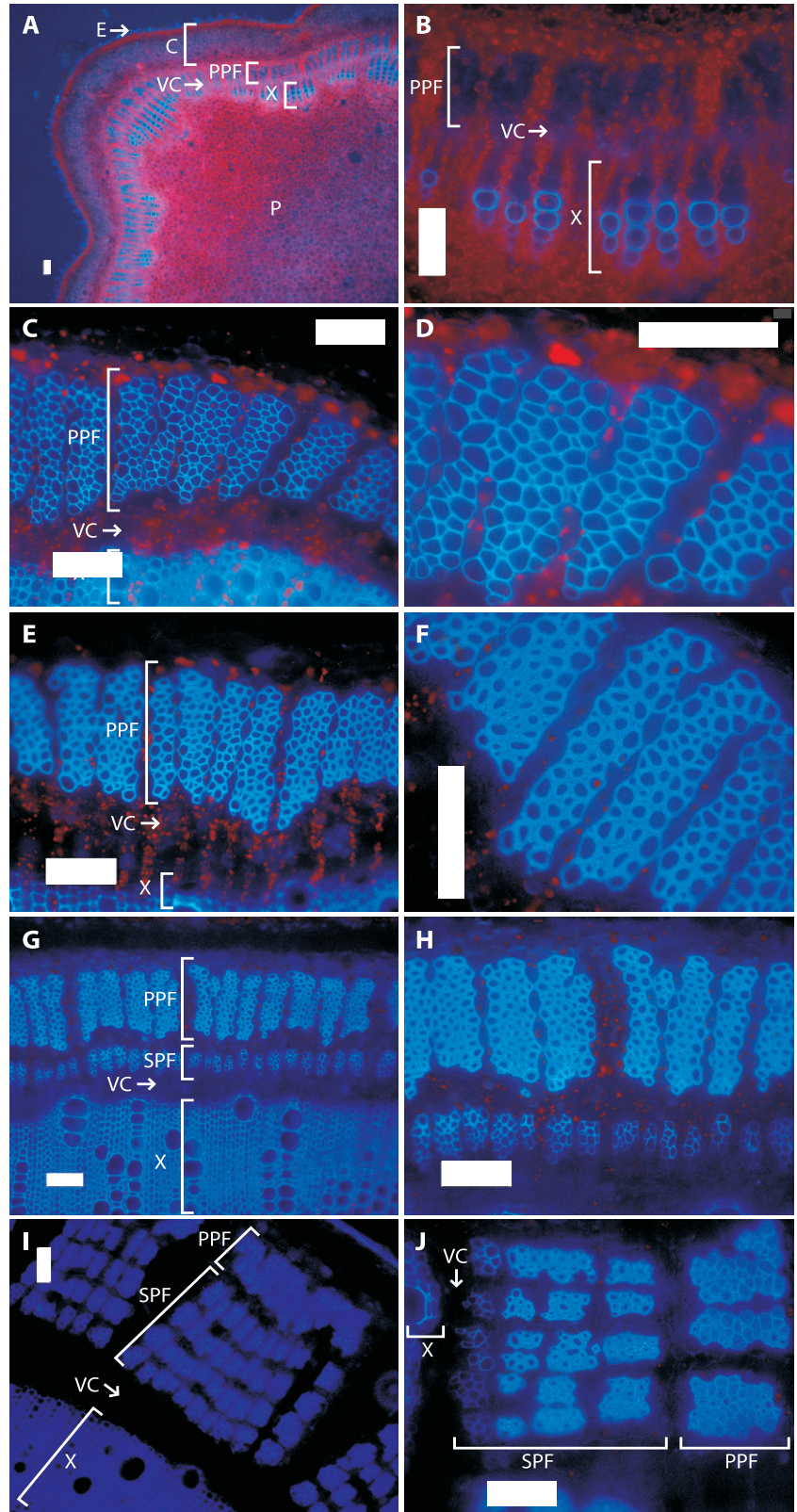


Figure 2 UV epifluorescence of kenaf stem cross-sections. (A) and (B) Stem cross-section of 90-day-old kenaf stem, 3.5 cm down from the shoot apex (B is higher magnification of A). (C) and (D) Stem cross-section 9.0 cm down from the shoot apex (D is higher magnification of C). (E) and (F) Stem cross-section 20.0 cm down from the shoot apex. (G) and (H) Stem cross-section 30.0 cm down from the shoot apex (H is higher magnification of G). (I) and (J) Stem cross-section 120.0 cm down from the shoot apex. The orientation of all pictures is that the epidermis is to the top or top right of the phloem fiber bundles, except A (oriented as labeled) and J (epidermis to the right). E, epidermis; C, cortex; PPF, primary phloem fiber; SPF, secondary phloem fiber; VC and arrow, vascular cambium; X, xylem; P, pith. All scale bars are 100 μm.

than natural retting, but increases cost [15, 16]. Chemical retting is rapid and consistent, and can separate individual fiber cells, but requires large quantities of caustic materials and consequently has negative environmental impacts.

Previous studies found that fiber length [18] and plant tissue composition [10, 19] differ in response to plant age and location along the stem. Here, the relationships among fiber development, morphology, and physical properties were examined. This study included a morphometric analysis of primary and secondary bast fiber bundles with respect to size and cell wall thickening. Bark ribbons were removed from different sections of the stem and naturally retted in water to obtain pools of fiber that differed in age, size, and wall thickness. The viscoelastic properties of the fibers in each pool were measured using a dynamic mechanical thermal analyzer (DMTA), operating in the tension mode, to probe both the elastic and viscous response of fiber bundles isolated from each pool [20, 21]. We found that primary fiber bundles had greater girth than secondary bundles, and that fiber-cell maturity contributed prominently to fiber viscoelastic properties. We note that the viscoelastic properties of polymer composites based on kenaf are being increasingly considered, and although the response of polymer matrices has been analyzed, the response of the fiber component has not been probed [22]. Understanding the relationship between the morphology and maturity of the fiber, and the fiber's viscoelastic properties, will contribute to development of future composites.

Materials and Methods

Growth of Kenaf and Fiber Isolation

Six to eight kenaf (*Hibiscus cannabinus* L., var. Tainung 2) plants per two gallon pot were grown from seed in Sun Gro Metro-Mix 366 (Bellevue, WA) supplemented with Scotts Osmocote Plus controlled release fertilizer (15-9-12) (Scotts-Sierra Horticultural Products Co., Marysville, OH) according to the manufacturer's instructions. Plants were maintained in a climate controlled greenhouse at 30 °C with automated watering twice daily for 30 minutes, and supplemental lighting with twelve 400 W metal halide bulbs on a 16/8 light/dark cycle. Stem length was measured at 60, 70, and 80 days post-planting to establish growth rate. Three 90-day-old stems were cut above the cotyledonary node; the cut surface of each stem was wrapped in wet paper towel and placed in a plastic bag for transport to the laboratory. The stems were then cut under water above the next node and kept in water at room temperature for the duration of the sampling. The leaves of each plant retained turgor during sampling, indicating that physiological levels of hydration were maintained.

Stem cross-sections (hand sections) were collected at various distances from the apices of each stem and observed by

bright field and UV epifluorescence (340 to 380 nm excitation, 435 to 485 nm emission) on a Nikon e600 compound microscope equipped with a DXM1200F camera (Nikon Instruments Inc., Melville, NY) and X-cite 120 Fluor System (Exfo Life Sciences Division, Mississauga, Canada). Wall thickening was quantified with ImageJ version 1.38x analytical software [23]. The stems were then cut in 30 cm-sections for retting. Each section was further cut to 10 cm and the bark layer containing the bast fiber was stripped from each section and completely submerged in distilled water in 1 liter Erlenmeyer flasks at room temperature. Ribbons of bark were brushed weekly with a bristle brush to remove loosened, non-fiber material, and returned to the flasks. Retting proceeded for five weeks. Retted fiber bundles were combed and characterized with bright field and UV epifluorescence. Statistical analysis was with Microsoft Excel (Redmond, WA).

Dynamic Mechanical Thermal Analysis of Kenaf Fibers

The viscoelastic properties of the retted fibers obtained from the different stem sections were measured using a DMTA (Rheometric Solids Analyzer 3, TA Instruments, New Jersey, USA), operating in the tension mode. In-phase storage and out-of-phase loss modulus were measured relative to temperature. Samples were scanned at a heating rate of 5 °C min⁻¹, at frequency of 1 Hz, and strain amplitude of 0.4% (determined from a separate strain amplitude sweep at 1 Hz) between 25 °C to 80 °C. Each material was run in triplicate and the average viscoelastic properties obtained. For each fiber population, three fiber bundles were isolated and diameters measured under an optical microscope. The gage length for all fibers was maintained at 25 mm, and the average diameters were 180.8 (±4.3), 175.6 (±5.5), 178.3 (±3.5), and 169.9 (±8.5) μm for the 30–60, 60–90, 90–120, and 120–150 cm from apex populations, respectively.

Results and Discussion

Kenaf Stem and Fiber Anatomy

When harvested for microscopy and retting, stems were 240 ± 7 cm long, and were growing 14.8 ± 3.8 cm every 10 days (n = 3, variation = standard deviation). In stem cross-sections, a ring of vascular cambium was evident 3.5 cm from the apex, indicating secondary growth had initiated (Figures 2A and 2B; whitish band indicated by the arrow). Files of xylem cells were evident internal to the vascular cambium, with older cells (more internal) showing prominent lignification (blue fluorescence). External to the vascular cambium, cells making up the phloem fiber bundles were most conspicuous by the lack of chlorophyll (i.e. lack

Table 1 Radial length (length in the radial dimension of the stem), width (length in the tangential direction of the stem), cross-sectional area, and number of cells in primary fiber bundles relative to secondary fiber bundles in kenaf stems. Primary fiber measurements are combined from cross-sections at 30 cm and 120 cm from the stock apex. Secondary fiber measurements are from cross-sections at 120 cm from the stock apex.

	Primary bundles	Secondary bundles	P, t-test
Radial length (μm)	205 \pm 33 (n = 24)	73 \pm 13 (n = 33)	< 0.001
Width (μm)	70 \pm 19 (n = 24)	47 \pm 11 (n = 33)	< 0.001
Area (μm^2)	1.14(10 ⁴) \pm 0.28(10 ⁴) (n = 24)	2.72(10 ³) \pm 0.90(10 ³) (n = 33)	< 0.001
Number of cells	45 \pm 10 (n = 34)	15 \pm 5 (n = 38)	< 0.001

Table 2 Total cross-sectional area of fiber bundles, total cell wall area, and percent of fiber bundle made up of cell wall. The remaining area in the bundles is primarily the central lumens or occasional intercellular spaces.

	Total area (μm^2)	Cell wall area (μm^2)	Percent cell wall
Primary bundle (30 cm)	1.15(10 ⁴) \pm 0.29(10 ⁴) (n = 21)	8.7(10 ³) \pm 2.2(10 ³)	75.6 \pm 2.4%
Developing secondary bundle (30 cm)	1710 \pm 900 (n = 26)	1010 \pm 550	58.0 \pm 8.2%
Mature primary bundle (130 cm)	7060 \pm 2140 (n = 25)	6610 \pm 2060	93.6 \pm 2.1%
Mature secondary bundle (130 cm)	2720 \pm 900 (n = 33)	2590 \pm 105	89.1 \pm 3.3%
Developing secondary bundle (130 cm)	1414 \pm 689 (n = 15)	861 \pm 504	57.4 \pm 12.7%

of red fluorescence) and modest lignification (faint blue fluorescence). These were primary fiber bundles differentiating from primary growth [9]. The cortex had collenchyma cells with abundant chloroplasts (red fluorescence), and the central pith contained large parenchyma cells. Near the apex of the plant, the pith parenchyma contained chloroplasts, but in older stem sections these appeared to be replaced by starch filled amyloplasts (not shown).

Nine cm from the apex, files of xylem formed a contiguous ring, and cells in the fiber bundles had well-defined, but relatively thin secondary cell walls, and prominent lumen (Figures 2C and 2D). Fiber wall thickening continued 20 cm and 30 cm from the apex, and the lumen area decreased proportionately (Figures 2E and 2F, and 2G and 2H, respectively). The fiber bundles were laterally separated by a layer of one to three parenchyma cells.

A new ring of fiber bundles was evident 30 cm from the apex, separated from the primary bundles by a layer of four to five cells that would include the phloem conducting cells and parenchyma cells [8]. This new ring of fiber bundles was derived from secondary growth at the vascular cambium, and therefore was a ring of secondary fiber bundles. Primary fiber bundles and secondary fiber bundles had distinct dimensions (Table 1). Relative to secondary bundles, the primary bundles were two to three times as long (in the radial dimension), moderately wider, contained three times as many cells, and had four times the cross-sectional area.

Under the growth conditions used, a new ring of secondary fiber bundles initiated from the vascular cambium

approximately every 30 cm from the growing apex (five bundles at 130 cm, Figures 2I and 2J; seven bundles at 210 cm, not shown), which corresponded to about three nodes. As expected, new layers of fiber and increased fiber maturity contributed to increased fiber yield. Stem sections 30 to 60, 60 to 90, 90 to 120, and 120 to 150 cm from the apex of the three stems retted yielded, respectively, 0.45, 0.93, 1.22, and 1.74 g of dry, retted fiber.

By comparing cell wall thickness in the primary bundles at 30 cm (Table 2; Figures 2G and 2H, compared to Figures 2I and 2J), it was clear that thickening continued as the bundles continued to age. This was also clear in the secondary bundles, where the more mature bundles towards the outside of the stem had thicker cell walls than the younger, more internal bundles (Table 2; Figures 2I and 2J). Within individual bundles, wall thickening appeared to be progressive, as suggested by the primary fiber in Figure 3E (older cells towards the top of the image). At maturity, the protoplasm of fiber cells often dies, and only the cell walls remain to provide structural support and protection [8]. Our analysis did not reveal the status of the protoplasm in the mature fibers, but it was clearly alive in the developing fibers since the protoplasm is required to provide cell-wall precursors that are transported to the extracellular space for wall assembly.

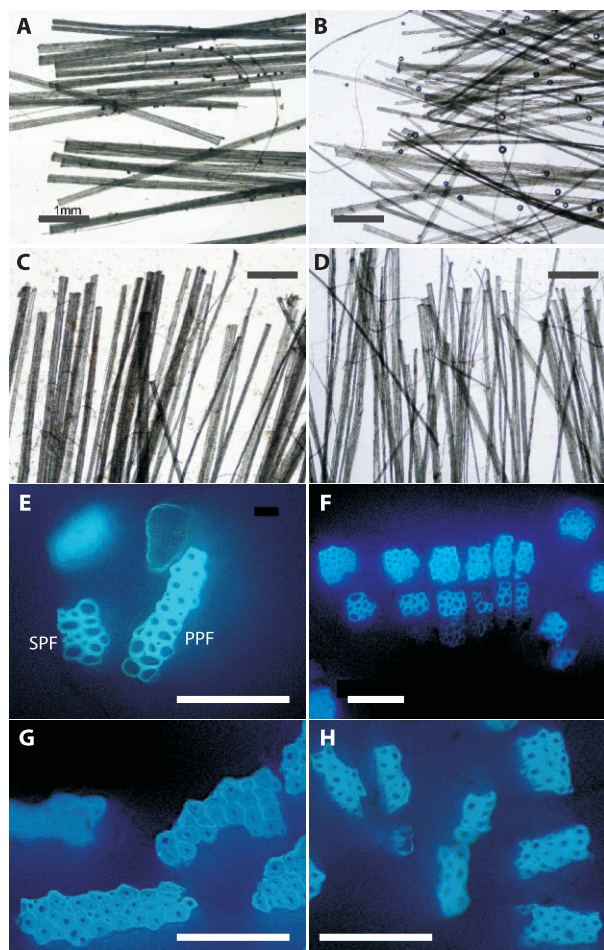


Figure 3 Retted fibers with bright field (A-D) and cross-sections with UV epifluorescence (E-H) microscopy. (A-D) Sample of fibers retted from stem sections 30 to 60 cm, 60 to 90 cm, 90 to 120 cm, and 120 to 150 cm, respectively. Scale bar, 1 mm. (E) UV epifluorescence of a maturing primary and secondary fiber bundle from the pool of fiber collected 60 to 90 cm below the shoot apex. Note the progression of cell wall thickening in the primary bundle from bottom to top. Note also that the bundles are fully retted and not connected or derived from the same stem section. PPF, primary phloem fiber; SPF, secondary phloem fiber. (F) Partially retted fiber bundles from the stem sections 90 to 120 cm below the shoot apex. The primary bundles are lost, and three layers of secondary bundles are present showing the progression from mature cell walls (top), thickening cell walls (middle), and secondary cell walls that have just initiated (bottom, faint blue fluorescence). (G) Mature primary fiber bundles from stem sections 120 to 150 cm from the shoot apex. (H) Mature secondary fiber bundles from stem sections 120 to 150 cm from the shoot apex. Scale bar, E-H, 100 μ m.

Viscoelastic Properties of Retted Fibers

Retting stem sections individually resulted in fiber populations enriched for fiber with different dimensions. Sections within 30 cm of the apices were not analyzed since they had no or very limited secondary thickenings. Sections 30 to 60 cm from the apices of the stems contained mostly the larger primary fibers (88% primary fiber, Table 3, Figure 3A), whereas sections from 120 to 150 cm contained primarily secondary fibers (82% secondary fiber, Table 3, Figure 3D). Intermediate stem sections had intermediate fiber proportions (Table 3, Figures 3B and 3C). In addition, water retting did not alter the morphology of the primary and secondary fibers, so the individual fiber populations had the same distribution of thick-walled mature fiber, and thin-walled developing fiber that was observed in the pre-retted, stem cross-sections (Figures 3E-3H).

Dynamic mechanical thermal analysis was performed on the different fiber pools to study their thermo-mechanical properties in the linear viscoelastic region. Table 4 shows that both E' and E'' increased with increasing distance from the shoot apex, and this correlated well with the wall thickening observed in the fiber bundles. The pool of fiber collected between 30 and 60 cm had the largest proportion of larger, primary fibers (Table 3), but fiber in this pool was also the least mature of that tested, and had the lowest E' and E'' values. The pool of fiber collected between 120 and 150 cm from the apex had the largest proportion of smaller, secondary fibers, but the largest proportion of mature, thick walled fiber, and the highest E' and E'' values. The intermediate pools of fiber had intermediate morphological parameters and viscoelastic properties. In particular, Table 4 shows that E' of the pool of fiber collected between 60–90 cm, 90–120 cm, and 120–150 cm increased to 3.19, 7.30, and 13.70 GPa, which was 56, 256, and 568% higher than that of the pool of fiber collected between 30–60 cm (2.05 GPa). Likewise, E'' increased by 211% (60–90 cm), 411% (90–120 cm), and 844% (120–150 cm) with respect to that of 30–60 cm from the apex.

Morrison et al. found that bast fibers within 15 cm of the shoot apex are not completely lignified, irrespective of plant age at harvest date [10]. This is consistent with our results, since fibers that were close to the growing tip were likely immature primary fibers with relatively thin walls. They also found that central stem sections of plants older than 60 days do not increase the proportion of lignin in bast fibers on a dry weight basis. At 60 days, our plants were 210 ± 3 cm, and stem sections halfway down from the apex had mature primary fiber bundles and one or two rings of mature secondary bundles. The findings of Morrison et al. are therefore also consistent with the morphological progression we observed; fibers were already heavily lignified and the addition of new lignin to developing bundles was likely proportional to the increase in fiber mass.

Table 3 Average width of fiber bundles retted from the indicated stem sections, and percentage of primary (> 100 µm wide) and secondary (< 100 µm wide) of the fiber making up the fiber pool.

Stem section, from shoot apex	Average fiber width (µm)	Percent primary fiber	Percent secondary fiber
30 – 60 cm	148 ± 28 ¹ (n = 26)	88	12
60 – 90 cm	95 ± 42 (n = 49)	37	63
90 – 120 cm	89 ± 29 (n = 42)	29	71
120 – 150 cm	79 ± 28 (n = 61)	18	82

¹ Standard deviation.**Table 4** Dynamic thermo-mechanical properties at 25 °C of the four fiber pools retted from different kenaf stem sections.

Stem section, from shoot apex	E' (GPa) 25 °C	E'' (GPa) 25 °C
30 – 60 cm	2.05 ± 0.05 ¹	0.09 ± 0.08
60 – 90 cm	3.19 ± 0.10	0.28 ± 0.05
90 – 120 cm	7.30 ± 0.07	0.46 ± 0.04
120 – 150 cm	13.7 ± 0.04	0.85 ± 0.06

¹ Standard deviation.

Conclusions

Bast fibers are bundles of thick-walled, phloem fiber cells which differentiate from thin-walled precursors. We are unaware of another study in kenaf that compares fiber bundle maturity and morphology in the growing plant with the viscoelastic properties of the retted product. Prior to complete maturation, bundles at different stages of development have different physical and chemical properties. By retting stem sections separately, we were able to measure fiber elasticity (E') and potential for energy absorption (out of phase loss modulus, E'') of fiber pools enriched for bundles of different maturity. Although younger stem sections had a greater proportion of larger, primary fibers, elasticity and damping capacity were less than fibers from older sections enriched for smaller secondary fibers and fibers with thicker cell walls. Cell wall thickness, rather than overall fiber dimensions, therefore appears to be a major determinant of properties that contribute to the utility of these fibers in industrial applications. While it would be expected that fiber stiffness would increase with maturity, we showed that the viscous response also increased. However, it is also notable that more mature fibers had reduced lumen area relative to younger fibers, and thus had different 'honeycomb' structural properties. For certain applications, the larger lumen of young fibers may impart a desirable characteristic. These results will help guide agronomic practices that favor distinct fiber characteristics, and could facilitate variety improvement for kenaf and other crops on the basis of functional properties in

addition to yield. These results will also help focus future genomic and biotechnology efforts to tissues in maturing stems most likely to improve yield and quality.

Acknowledgements

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